How are trace elements managed in the cells of *Chaoborus* larvae to avoid ill-effects?

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ABSTRACT

Larvae of the phantom midge *Chaoborus* have been proposed as sentinels to estimate the bioavailable concentrations of trace elements. Because they can tolerate acidic, metal-contaminated, environments, in which many other sentinels cannot live, they offer an opportunity to investigate the metal-handling strategies that allow them to prosper. Using *Chaoborus* larvae collected from lakes impacted by metals, we first determined the subcellular partitioning of Cd and Ni. The majority (up to 70%) of the Cd and Ni in larvae was detoxified by thermo-stable, cytosolic ligands. We then screened these metal-ligand complexes by SEC-ICP. The results revealed that cytosolic ligands with a similar molecular weight to metallothioneins (4-5 kDa) of the fruit fly (*D. melangonaster*) are important for Cd detoxification, whereas for Ni handling other ligands differing in their molecular weights (<10 kDa) are involved.

INTRODUCTION

Chaoborus larvae are useful as biomonitors of trace elements in lakewater and plankton…

- easy to collect and identify to species
- abundant and widely distributed
- tolerate wide pH range
- their [Cd], [Ni] and [Se] are proportional to those in water and plankton
- tolerate high concentrations of trace elements

QUESTIONS -- OBJECTIVES

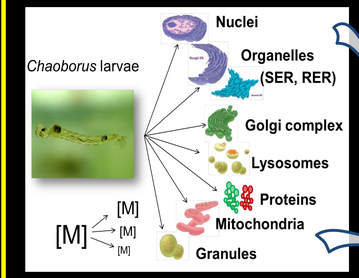
1. How do *Chaoborus* "manage" Cd and Ni?
   To measure Cd and Ni partitioning in the cells of *Chaoborus* collected from lakes differing in their concentrations of these metals

2. How do *Chaoborus* detoxify Cd and Ni?
   To determine the molecular weights of the bioligands to which Cd and Ni are bound in the cytosol of *Chaoborus* cells (metallomics)

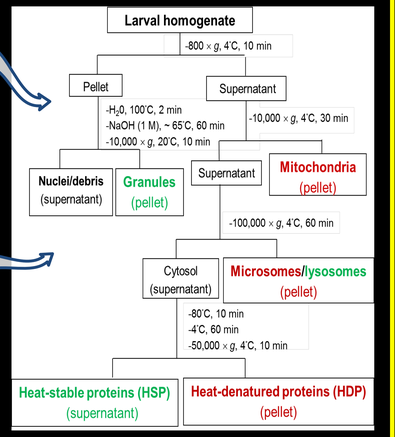
METHODS

1. Cd and Ni partitioning in cells

Subcellular fractions



Protocol

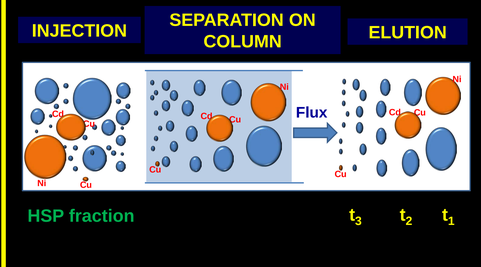


Detoxified metal fractions:
-Heat-stable proteins (HSP)
-Granules

Metal-sensitive fractions:
-Heat-denatured proteins (HDP)
-Mitochondria
-Microsomes/lysosomes

2. Metallomics of Cd and Ni in HSP fraction

Size exclusion chromatography (SEC) on line ICP-MS



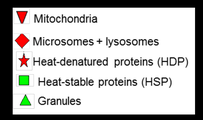
Sample volume: 100 µL
Buffer: 100 mM ammonium acetate (pH=8.0)
Flow rate: 0.7 mL/min
System: Agilent 1260 infinity Series
Detector: UV at 280 nm and 254 nm
Columns : Superdex Peptide 10/300 GL

System: Agilent 7500
Cones: Platinum cones
Detection: ^{60}Ni, ^{114}Cd

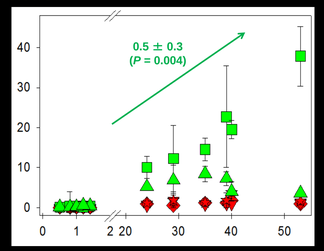
RESULTS

1. Cd and Ni partitioning in cells

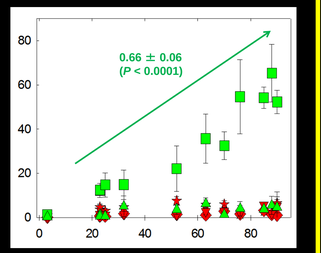
Mean ± SD, 12 lakes
n = 5 (30 larvae/sample)



[Ni] (nmol g^{-1}) fraction



[Cd] (nmol g^{-1}) fraction



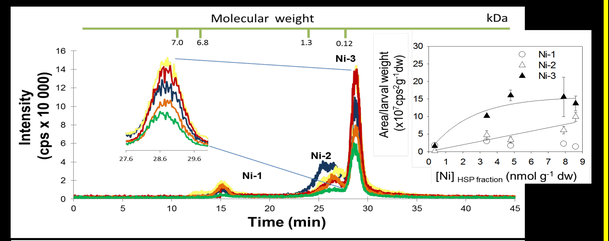
[Ni] *Chaoborus* (nmol g^{-1})

[Cd] *Chaoborus* (nmol g^{-1})

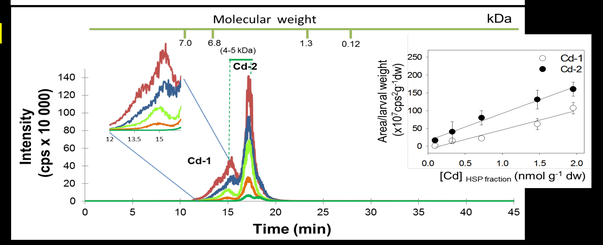
2. Metallomics of Cd and Ni in the HSP fraction

HSP fractions, 5 lakes
n = 3 (30 larvae/sample)
each lake is represented by one-color line

^{60}Ni



^{114}Cd



CONCLUSIONS

Larvae of *Chaoborus* handle Cd and Ni by binding them to cytosolic and heat-stable ligands (HSP)".

Cd and Ni are also accumulated in putative metal-sensitive fractions, which suggests that metal detoxification is incomplete.

Cd and Ni in the HSP fraction are not bound to the same molecular-weight pool (Cd: metallothionein-like proteins; Ni: <10 kDa proteins)

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